MOLECULAR AND CELLULAR BIOLOGY, Sept. 1993, p. 5918–5927
0270-7306/93/095918-10$02.00/0

Vol. 13, No. 9

In Vitro Transcription of a *Drosophila* U1 Small Nuclear RNA Gene Requires TATA Box-Binding Protein and Two Proximal *cis*-Acting Elements with Stringent Spacing Requirements

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Received 12 April 1993/Returned for modification 25 May 1993/Accepted 4 June 1993

Transcription of a *Drosophila* U1 small nuclear RNA gene was functionally analyzed in cell extracts derived from 0- to 12-h embryos. Two promoter elements essential for efficient initiation of transcription in vitro by RNA polymerase II were identified. The first, termed PSEA, is located between positions −41 and −61 relative to the transcription start site, is crucial for promoter activity, and is the dominant element for specifying the transcription initiation site. PSEA thus appears to be functionally homologous to the proximal sequence element of vertebrate small nuclear RNA genes. The second element, termed PSEB, is located at positions −25 to −32 and is required for an efficient level of transcription initiation because mutation of PSEB, or alteration of the spacing between PSEA and PSEB, severely reduced transcriptional activity relative to that of the wild-type promoter. Although the PSEB sequence does not have any obvious sequence similarity to a TATA box, conversion of PSEB to the canonical TATA sequence dramatically increased the efficiency of the U1 promoter and simultaneously relieved the requirement for the upstream PSEA. Despite these effects, introduction of the TATA sequence into the U1 promoter had no effect on the choice of start site or on the RNA polymerase II specificity of the promoter. Finally, evidence is presented that the TATA box-binding protein is required for transcription from the wild-type U1 promoter as well as from the TATA-containing U1 promoter.

Small nuclear RNAs (snRNAs) are involved in processing primary transcripts of mRNAs and rRNAs. For example, the small nuclear ribonucleoprotein particles that contain the U1, U2, U4, U5, and U6 snRNAs are involved in pre-mRNA splicing (12, 49). Although the genes coding for the U1 to U5 snRNAs are transcribed by RNA polymerase II (Pol II), the promoters of these genes in vertebrates lack TATA boxes. Instead, a proximal sequence element (PSE), located at approximately positions −50 to −60 in the 5′-flanking DNA, is required for a basal level of transcription and for specifying the correct transcription initiation site (7, 34, 37). The PSE is also necessary to establish a functionally unique snRNA transcription complex that is capable of recognizing a 3′ end formation signal in the 3′-flanking DNA of vertebrate snRNA genes (14, 15, 32, 33).

Unlike the other snRNAs, U6 snRNA is synthesized by RNA polymerase III (Pol III). Surprisingly, a TATA box located near position −30 is a dominant element for determining Pol III specificity of vertebrate U6 snRNA genes (8, 24, 25, 29). Transcription from U6 promoters is additionally dependent upon a PSE that is structurally homologous and functionally interchangeable with the PSE of the Pol II snRNA genes of vertebrates (8, 24, 29). However, the location of the PSE relative to the start site differs slightly between the Pol II and U6 snRNA genes, and this positioning serves as an additional determinant of Pol III specificity (22, 26).

Although U6 snRNA genes can be accurately transcribed by Pol III in vitro with a fairly high degree of efficiency (24, 36, 52), transcription of vertebrate Pol II snRNA genes in cell extracts has historically proven to be difficult. Systems reported up to the present time are still relatively inefficient (11) or require manual dissection of germinal vesicles from *Xenopus* oocytes (28). Sea urchin snRNA genes can be transcribed in vitro (30, 46), but the extracts and general transcription factors from sea urchins are less well characterized than those in mammalian systems. As a result of these difficulties, progress in the fractionation and biochemical characterization of factors involved in Pol II snRNA transcription has been slow.

Over the last several years, the *Drosophila melanogaster* system has proven to be an excellent source of nuclear extracts that are highly active in transcription by Pol II (13, 18, 45). *Drosophila* nuclear extracts have also been subjected to intensive biochemical fractionation and characterization, and in general, the basal transcription factors are interchangeable with the homologous mammalian factors (10, 53). Recently, Kamakaka et al. (19) showed that a *Drosophila* U1 snRNA gene could be accurately and efficiently transcribed in vitro by Pol II in a soluble nuclear fraction (SNF) prepared from 0- to 12-h embryos. We have been using this system to further analyze transcription of the *Drosophila* U1 snRNA gene in vitro.

Our studies, reported here, indicate that two distinct sequence elements, evolutionarily conserved in the 5′-flanking DNA of *Drosophila* U1, U2, and U4 snRNA genes (1, 21, 23, 39), are required for efficient transcription of the U1 gene in vitro. The more distal element (PSEA, positions −41 to −61), is essential for detectable U1 transcription and is the dominant element for specifying the transcription initiation site. On the basis of its location and function, PSEA is apparently homologous to the vertebrate PSE. The more

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proximal element (PSEB, positions −25 to −32) significantly contributes to the efficiency of transcription. Although PSEB occupies a position normally occupied by a TATA box, PSEB does not closely resemble the consensus TATA box sequence. Interestingly, when PSEB was replaced with a consensus TATA sequence, the basal level of transcription increased fourfold over that of the wild-type U1 promoter. Moreover, Pol II specificity was maintained, and the promoter functioned independently of the upstream PSEA.

Recent results from a number of laboratories have indicated that all three eukaryotic RNA polymerases require the TATA box-binding protein (TBP) for transcription of both TATA-containing and TATA-less mRNA, rRNA, U6 RNA, and tRNA promoters (38). Here we present evidence that TBP (and associated factors) is also required for initiation of transcription by Pol II on the U1 snRNA promoter. Together, the results reported here indicate that the *Drosophila* system should be extremely useful for purifying and characterizing factors involved in Pol II snRNA transcription.

MATERIALS AND METHODS

Plasmid templates for in vitro transcription. The wild-type template (DU1WT) contains 5′-flanking DNA and coding sequences of the *Drosophila* U1 95.1 gene extending from position −391 to +32 cloned into the pUC118 vector; it is identical to the plasmid previously termed pUC-U1a (19). Templates DU1Δ-32, DU1Δ-72, DU1Δ-126, DU1Δ-183, DU1Δ-215, and DU1Δ-288 were similar but truncated in the 5′-flanking DNA at *Sty*I, *Mlu*I, *Fsp*I, *Msc*I, *Ssp*I, or *Bsp*EI restriction sites, respectively. First-generation plasmids that contained nucleotide substitutions were generated by cloning synthetic double-stranded DNA between unique restriction sites in the DU1WT template (e.g., *Mlu*I at −72, *Sty*I at −32, and *Xba*I in the pUC118 polylinker downstream of the U1 coding sequence). In many cases, these substitutions were used to incorporate new unique restriction sites that facilitated subsequent construction of second-generation plasmids that contained additional substitutions and insertions introduced via similar cloning methods. DU1i2 and DU1d1 were created by digestion of DU1i3 and DU1s6, respectively, with *Bst*EII and treating the resulting fragments with S1 nuclease to remove the 5-base overhangs, followed by religation of the products. DU1i4 was constructed by digesting DU1i3 with *Bst*EII, filling in the ends, and recircularizing. All plasmids used in this study were purified by two CsCl gradient centrifugations, and the promoter mutations were confirmed by dideoxy sequencing.

In vitro transcription assays. In vitro transcription and analysis of transcription products by primer extension were performed essentially as described by Kadonaga (18) and Kamakaka et al. (19). Transcription reaction mixtures (25-μl final volume) contained 10 μl of *Drosophila* embryo SNF, 5.5 μl of HEMG buffer (25 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid] [pH 7.6], 12.5 mM $MgCl_2$, 0.1 mM EDTA, 10% glycerol, 1.5 mM dithiothreitol) containing 0.1 M KCl, 7.5 μl of ribonucleoside triphosphate (rNTP) mix (1.7 mM each rNTP in 0.67 M HEPES [pH 7.6]), and 2 μl of plasmid DNA (0.1 mg/ml in 10 mM Tris [pH 8.0]–1 mM EDTA). Modifications specific to individual experiments are described in the figure legends. Following a 1-h incubation at room temperature, transcription was stopped by adding 105 μl of a solution containing 0.25 mg of glycogen per ml, 20 mM EDTA (pH 8), 0.2 M NaCl, 1% sodium dodecyl sulfate (SDS), and 120 μg of proteinase K per ml; incubation was then continued for 3 to 5 min. Approximately 1,000 cpm of an end-labeled 64-bp double-stranded oligonucleotide or of an end-labeled 54-mer single-stranded oligonucleotide was added to each tube as a recovery standard. After extraction with phenol-chloroform and precipitation with ethanol, the transcripts were annealed to a reverse transcription primer (500,000 cpm, labeled with polynucleotide kinase and [γ-^{32}P]ATP) for 1 h at 45°C. The primer was either a commercially available 17-mer oligonucleotide complementary to pUC118 downstream of the polylinker (New England Biolabs no. 1211) or a similar primer (24-mer) that contained 7 more nucleotides of complementary sequence at the 5′ end. Correct initiation of transcription at the U1 start site resulted in primer extension products of 82 or 89 nucleotides in length, respectively. Primer extension was performed with 200 U (1 μl) of RNase H$^-$ Moloney murine leukemia virus reverse transcriptase (Bethesda Research Laboratories) per reaction. The products of the primer extension were separated in 10% denaturing polyacrylamide gels and subjected to autoradiography at −70°C. To calculate relative transcriptional activity, additional aliquots of the samples were run in gels with lanes skipped between samples (not shown). Following autoradiography, bands corresponding to the U1 primer extension products and the recovery standard were cut out, and radioactivity was determined by scintillation counting.

Oligonucleotides for sequestering TBP. When added to an in vitro transcription reaction mixture, oligonucleotides that contain the TATA box sequence can inhibit transcription by binding and sequestering TBP (55). When we initially attempted such inhibition experiments with the *Drosophila* system by using linear double-stranded oligonucleotides, no inhibition was observed because of rapid degradation of the oligonucleotides by potent exonucleases present in the *Drosophila* nuclear extract (reference 44 and our unpublished results). To circumvent this problem, ligated circular "dumbbell" oligonucleotides that are resistant to exonuclease digestion were prepared (4). A double-stranded dumbbell oligonucleotide containing the adenovirus major late promoter TATA box region was prepared from the synthetic oligonucleotides 5′-GGGGCTATAAAAGGGGGTGGC TActttcTAGCCA-3′ and 5′-CCCCCTTTTATAGCCCCCCT GCctttcGCAGG-3′. The oligonucleotides were individually phosphorylated with T4 polynucleotide kinase, annealed in equimolar quantities, and intramolecularly ligated with T4 DNA ligase. The closed circular oligonucleotide, which has been ligated at two positions and migrates faster than the once-ligated product of the same size, was purified by electrophoresis in denaturing 10% polyacrylamide gels. The resultant dumbbell oligonucleotide contained the wild-type DNA sequence of the adenovirus major late promoter from position −39 to −17 flanked by 2 (5′ end) or 3 (3′ end) bp of nonspecific sequence and by a 5-nucleotide single-stranded loop (shown above in lowercase letters) at each end. A mutant TATA dumbbell oligonucleotide was prepared in parallel from the oligonucleotides 5′-GGGGCTAGAGAAG GGGGTGGCTActttcTAGCCA-3′ and 5′-CCCCCTTCTCT AGCCCCCCTGCctttcGCAGG-3′. The resultant mutant oligonucleotide was identical to the one described above except for two base changes in the TATA box (underlined) that abolish binding to TBP (17). These particular oligonucleotides were selected on the basis of the work of White et al. (55).

Additional reagents. *Drosophila* TBP (dTBP) was produced in *Escherichia coli* and purified as described by Hoey et al. (16) with the substitution of 0.01% for 0.1% Nonidet P-40 in the buffers. As an additional purification step, the 0.4

M KCl peak from the heparin column was dialyzed against HEMG buffer plus 0.1 M KCl–0.01% Nonidet P-40 and then applied to a Mono S column (Pharmacia) equilibrated with 5 volumes of the same buffer. Following sample application, the column was eluted with a 16-ml gradient of 0.1 to 1.0 M KCl. The active protein, which eluted at 0.35 M KCl, was dialyzed against HEMG buffer plus 0.1 M KCl. Antibodies against TBP were prepared in rabbits by using the 1.0 M KCl fraction from the heparin column as the immunogen. The immunoglobulin G fraction was then twice batch purified from the antiserum by using Bakerbond ABx resin (J. T. Baker, Philipsburg, N.J.) according to the manufacturer's directions. The purified immunoglobulin G was then dialyzed against HEMG buffer plus 0.1 M KCl and was estimated to be 70 to 80% pure as assayed by Coomassie staining of SDS-polyacrylamide gels. The *Drosophila* TFIID fraction was prepared as described by Wampler et al. (53). Recombinant human TBP (hTBP) was purchased from Promega.

When added, α-amanitin (Sigma) was included in transcription reaction mixtures at a final concentration of 2.5 μg/ml. Tagetitoxin (Tagetin; Epicentre Technologies, Madison, Wis.), a specific inhibitor of Pol III (48), was added at a final concentration of 200 U/ml.

RESULTS

Basal U1 transcription in vitro requires DNA sequences upstream of position −32. Kamakaka et al. (19) recently showed that 391 bp of 5′-flanking DNA and 32 bp of U1 coding sequence were sufficient to direct accurate and efficient transcription of a *Drosophila* U1 snRNA gene in vitro. As a first step toward characterizing the *Drosophila* U1 gene promoter, we initially constructed the 5′-truncation templates shown at the bottom of Fig. 1 and assayed these templates by using the in vitro transcription system. The results of primer extension assays measuring the relative transcription efficiencies are shown in Fig. 1. Constructions truncated at positions −288, −215, −183, and −126 were each expressed at a level similar to that of the wild-type template (Fig. 1, lanes 1 to 5). A construction truncated at position −72 exhibited a 2.5-fold reduction in template activity compared with the wild type (Fig. 1, lane 6). When the template retained only 32 bp of 5′-flanking DNA, transcription was abolished (Fig. 1, lane 7). These results indicate that DNA sequences 32 to 72 bp upstream of the coding region are required for initiation of transcription from the U1 promoter in vitro. Sequences farther upstream between positions −72 and −126 appear to have a 2.5-fold stimulatory activity; the DNA sequences responsible for this mild stimulatory activity have not been further investigated in the current study. Moreover, since our transcription assays were performed in vitro, enhancer-type elements farther upstream may have gone undetected in our analysis.

Functional importance of PSEA, PSEB, and the initiation site for U1 transcription in vitro. To dissect further the functional elements in the proximal region of the U1 promoter, templates containing blocks of point mutations scanning through the region −68 to +8 were constructed (Fig. 2). Results of transcriptional analyses indicated that nucleotide changes upstream of, between, or downstream of the conserved PSEA and PSEB elements had relatively minor (less than twofold) effects on the level of U1 transcription in vitro (Fig. 2, templates U1s1, -s5, -s6, -s8, -s9, -s10, and -s11). However, clusters of mutations anywhere within PSEA essentially eliminated detectable transcription (templates

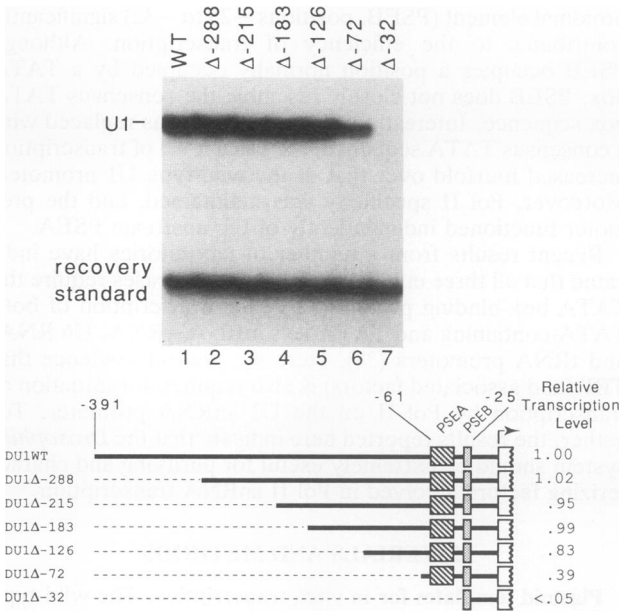

FIG. 1. In vitro transcription of a *Drosophila* U1 snRNA gene: 72 bp of 5′-flanking DNA is sufficient for a basal level of promoter activity. Circular plasmid DNA templates that contained various amounts of U1 gene 5′-flanking DNA were transcribed in an SNF prepared from *Drosophila* embryos, and the transcription products were assayed by primer extension. The band corresponding to the 82-nucleotide extension product arising from correct initiation of transcription of the U1 gene is labeled U1. A band corresponding to a 64-mer recovery standard is also indicated. The U1 templates, truncated at various positions in the 5′-flanking DNA, are schematically diagrammed at the bottom of the figure. The locations of PSEA, PSEB, and the beginning of the U1 coding region are indicated. The relative template activity of each construction is shown in the column at the right.

U1s2, -s3, and -s4), and mutation of the PSEB element to a GC-rich sequence reduced transcription eightfold (U1s7). Interestingly, a 3-base change that converted the PSEB sequence to a consensus TATA box (U1TATA; Fig. 2, lane 12) resulted in a 4.5-fold increase in transcription relative to that of the wild-type template. Experiments further investigating this effect are described in a later section of this report. Base substitutions spanning the transcription start site (−5 to +8) had no detectable effect on the efficiency of transcription (U1s11, lane 16), but the reverse transcription product resulting from this template reproducibly appeared to be about 1 nucleotide shorter than normal, suggesting that the sequence in this region may influence the choice of start site. This is probably due to the fact that Pol II exhibits some degree of site preference for initiation of transcription. In summary, the data shown in Fig. 2 indicate that PSEA is an essential element for U1 transcription and that the sequence of PSEB can have dramatic effects on the efficiency of initiation in vitro.

PSEA is the dominant element for specifying the transcription initiation site, but spacing between elements is crucial for efficient expression in vitro. Since two conserved elements, PSEA and PSEB, are required for efficient transcription, we next investigated the effects of altering the spacing between elements in the U1 promoter. First, we inserted 10 bp of DNA either between PSEB and the normal initiation site or between PSEA and PSEB (Fig. 3C, templates U1i1 and

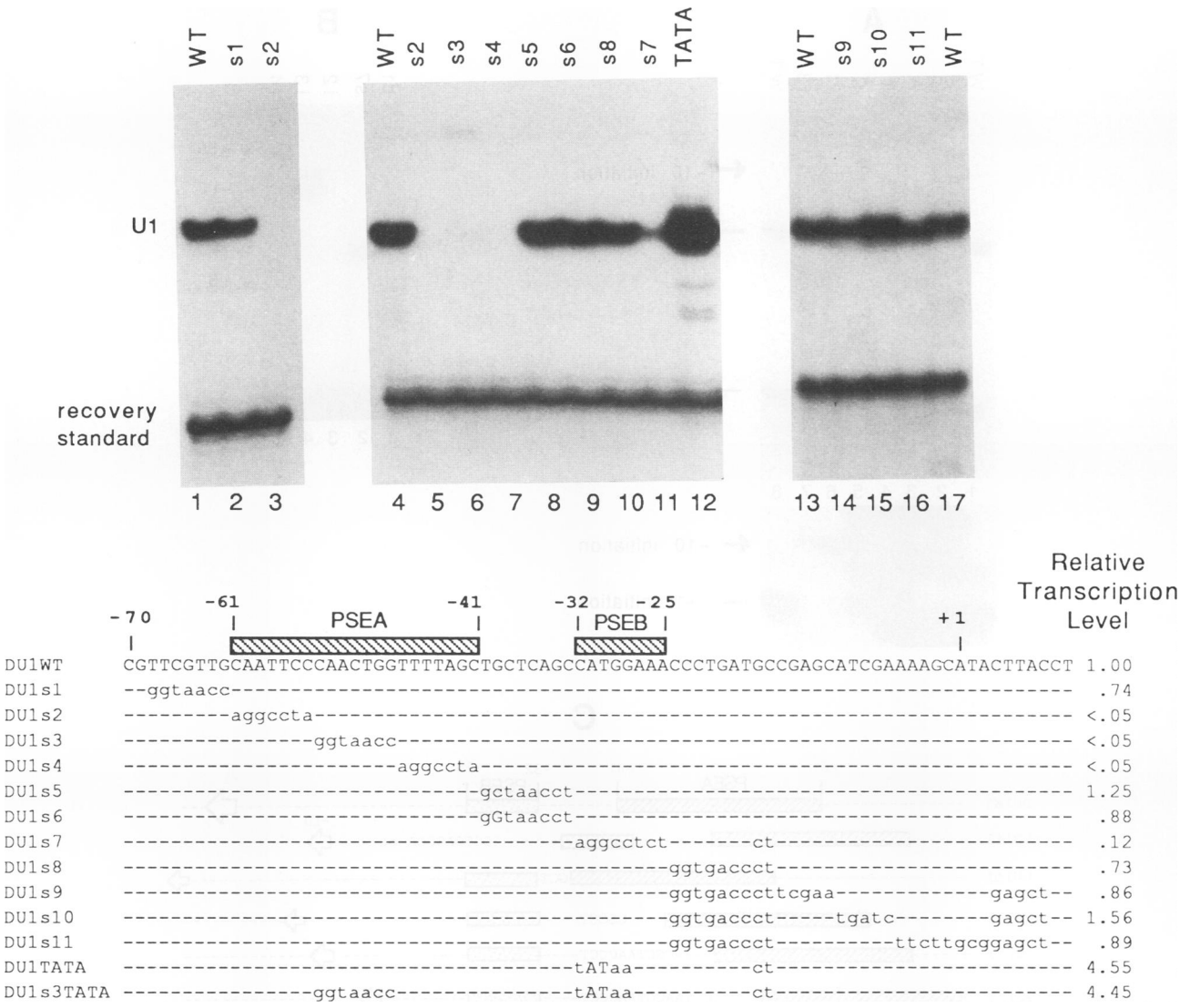

FIG. 2. Localization of the *cis*-acting elements required for U1 transcription in vitro. Templates with nucleotide substitutions scanning through the U1 promoter region from position −68 to +8 were constructed and assayed for transcriptional activity as described in Materials and Methods and the legend of Fig. 1. The sequence of the wild-type template from position −72 to +10 is shown in the top line in the lower part of the figure. The regions that correspond to the conserved PSEA and PSEB elements are designated by the striped bars. In the mutant constructions shown at the bottom, dashed lines indicate nucleotides identical to those in the wild type, and lowercase letters indicate the substitutions that were introduced. The relative transcription level of each template is shown in the column at the right and is an average from at least two separate experiments.

U1i3, respectively). Insertion of 10 bp between PSEB and the U1 coding region resulted in the initiation of transcription 10 bp upstream of the normal start site, although at a threefold-reduced level (Fig. 3A, lane 6). When 10 bp was inserted between PSEA and PSEB, a longer exposure of the gel was required to visualize the transcription product (Fig. 3A, bottom panel, lane 7), but the major initiation site was again shifted to position −10 (with a minor initiation at about −12). Together, these results indicate that the dominant *cis*-acting element for determining the transcription initiation site is PSEA, not PSEB or the sequence surrounding position +1. However, the relative level of transcription from the 10-bp insertion templates was reduced either 3- or 10-fold in comparison with that of the wild type, suggesting that the efficiency of transcription is partially dependent upon the spacing or alignment of the *cis*-acting elements along the DNA.

To examine this further, templates with an insertion of 5 or 15 bp or a deletion of 5 bp between PSEA and PSEB were constructed (Fig. 3C). All of these templates (U1i2, -i4, and -d1) were severely deficient in template activity (>30-fold reduction). Upon a very long exposure, light bands corresponding to very weak initiations occurring at approximately a constant distance (~40 bp) downstream of the PSEA sequence were visible (Fig. 3B). These results provide further evidence that PSEA is the dominant element for specifying the transcription initiation site in vitro, but the spacing of promoter elements is also crucial for normal levels of gene activity. Interestingly, the template with the 10-bp insertion, which maintained the phasing of the two

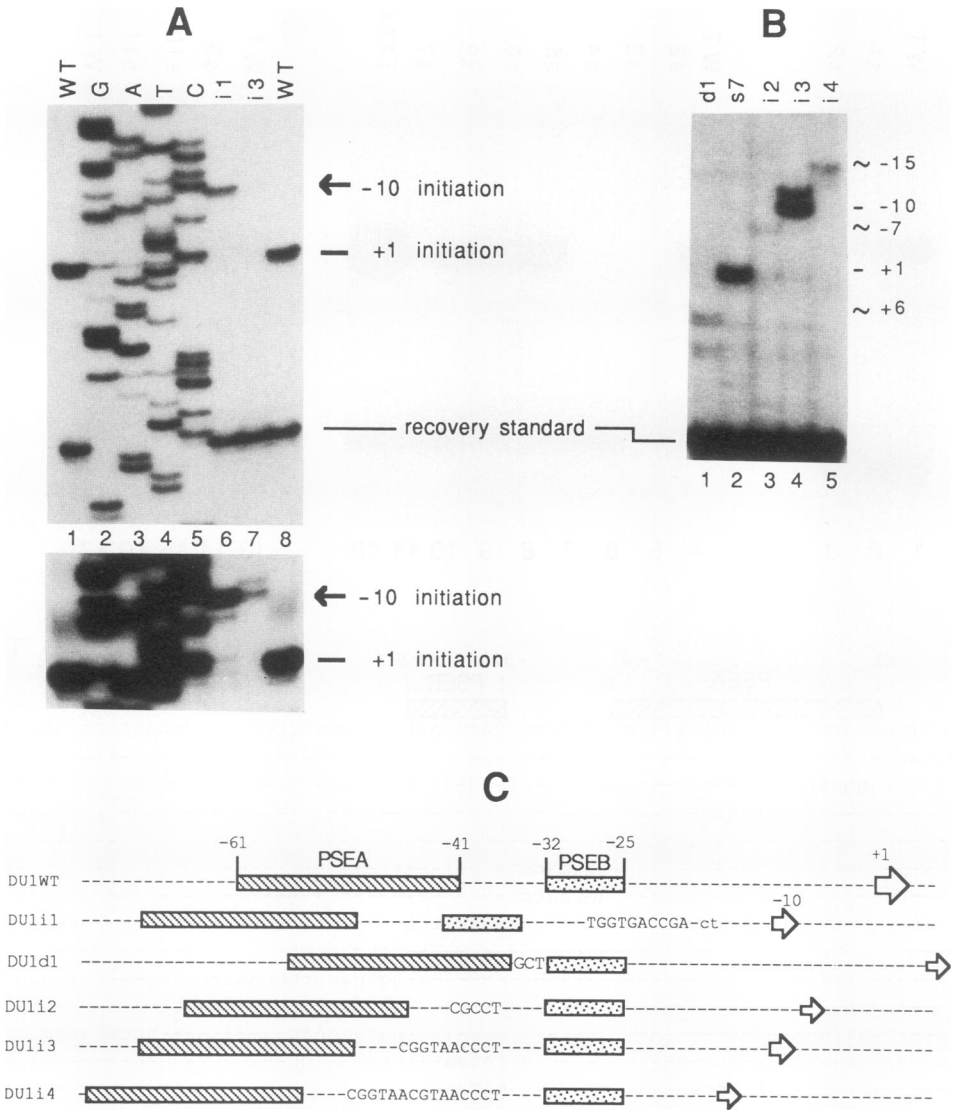

FIG. 3. Alteration of the distances between PSEA, PSEB, and the U1 coding region affects transcription efficiency and selection of the start site. (A) Either the wild-type template or templates containing 10-bp insertions between PSEA and PSEB (U1i3) or between PSEB and the U1 coding region (U1i1) were transcribed in vitro, and the primer extension products were run alongside sequencing ladders generated by using the same primer and the wild-type template. The two templates that contained 10-bp insertions at different positions each initiated transcription 10 bp upstream of the +1 initiation site; since the U1i3 template is transcribed at about only 10% of the wild-type level, the band is visible only upon an extended exposure (lower panel). The upper and lower panels represent 1- and 7-day exposures, respectively, of the same gel. (B) Templates with a 5-bp deletion (U1d1) or 5-, 10-, and 15-bp insertions (U1i2, -i3, and -i4, respectively) between PSEA and PSEB were transcribed and assayed by primer extension. Note that the autoradiogram shown is a very long (10-day) exposure and that the U1s7 (Fig. 2) and U1i3 templates, which are expressed at about 10 to 12% of the wild-type level, serve as size markers for +1 and −10 initiations, respectively. Bands corresponding to very weak initiations at about positions +6, −7, and −15 for the U1d1, -i2, and -i4 templates, respectively, are indicated. (C) Structures of the insertion and deletion templates in comparison with the wild type. Uppercase letters indicate extra nucleotides present in the insertion mutants or the three nucleotides that remain between PSEA and PSEB in the deletion mutant. The lowercase letters (ct) in U1i1 are nucleotide substitutions introduced for cloning purposes. Arrows indicate the location of the primary site of transcription initiation in each template.

promoter elements on the DNA helix, worked considerably better than the templates with 5- or 15-bp insertions. This suggests that there may be direct communication between factors bound to the two elements.

Conversion of PSEB to a consensus TATA element does not affect the Pol II specificity of the *Drosophila* U1 promoter but increases its efficiency and renders it independent of PSEA. The PSEB element in the U1 promoter is located at a position normally occupied by a TATA box in mRNA and U6 snRNA promoters. Although PSEB has little sequence similarity to a TATA box, it can be converted to a consensus TATA sequence by three nucleotide transitions (CATGGA to TATAAA). When these changes were made (template U1TATA), transcription was correctly initiated at position +1, but at a 4.5-fold-higher level than that from the wild-type U1 template (compare lanes 4 and 12 in Fig. 2 and lanes 1

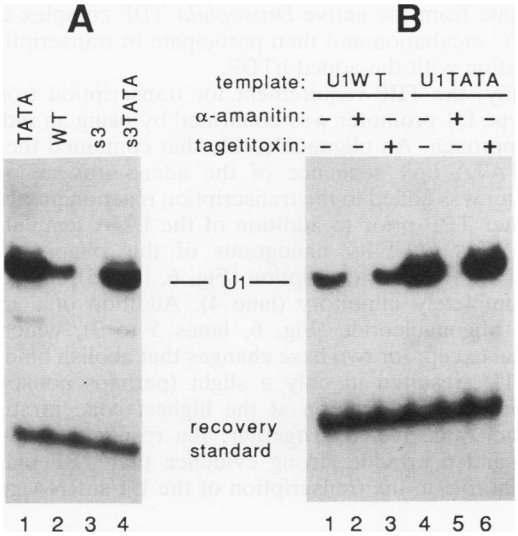

FIG. 4. Effect on promoter activity of converting PSEB to a canonical TATA sequence. (A) In vitro transcription reactions comparing the promoter activities of U1WT, U1TATA, U1s3, and U1s3TATA constructions. The sequences and relative transcriptional activities of the constructions are shown in the lower part of Fig. 2. Note that the s3 mutation inactivates PSEA. (B) The U1WT and U1TATA templates are both transcribed in vitro by Pol II. Pol II activity was inhibited by adding 2.5 μg of α-amanitin per ml to the transcription reaction mixtures shown in lanes 2 and 5. To inhibit Pol III, tagetitoxin was included at a concentration of 200 U/ml in the reaction mixtures shown in lanes 3 and 6.

and 2 in Fig. 4A). Interestingly, a mutation (U1s3) that inactivated PSEA and abolished U1 transcription in the context of the wild-type PSEB (Fig. 4A, lane 3) had no effect on transcription when combined with the canonical TATA box sequence (lane 4). In addition, a template with a 10-bp insertion between PSEA and the TATA box (a combination of U1i3 and U1TATA) also initiated transcription at position +1 with an efficiency 4.5-fold greater than that of the wild type (data not shown). Together, these results indicated that conversion of PSEB to a canonical TATA box allowed the promoter to function independently of PSEA.

We next considered the possibility that introduction of the consensus TATA sequence converted the *Drosophila* U1 promoter to Pol III specificity, as has been observed in several instances for vertebrate snRNA genes (24, 29). To test this, transcription reactions on the U1WT and U1TATA promoters were carried out in the presence of specific inhibitors of Pol II and III. When a low concentration of α-amanitin (2.5 μg/ml) was included in the reaction mixtures, transcription from the U1WT and U1TATA templates was inhibited (Fig. 4B, lanes 2 and 5), indicating that both templates were transcribed by Pol II. Conversely, when tagetitoxin, a specific inhibitor of Pol III (48), was added to the reaction mixtures, transcription from either template was unaffected (Fig. 4B, lanes 3 and 6). (In other experiments, not shown, transcription of a U6 snRNA template was severely inhibited by this same concentration of tagetitoxin.) In summary, the U1TATA and U1WT templates both were transcribed by Pol II, but they differed in terms of basal efficiency and their requirement for the PSEA element. Whether the U1TATA promoter functions as an snRNA-specific promoter or as an mRNA-type promoter is as yet unknown. In either case, it serves as a useful control promoter in the TBP depletion studies described below.

U1 transcription in vitro requires TBP. We next examined whether TBP is required for transcription from the U1 promoter. To do this, *Drosophila* embryo nuclear extract was preincubated with increasing amounts of antibodies made to bacterially expressed dTBP (α-dTBP antibodies). This extract was then used in transcription reactions with the U1WT and U1TATA constructions. The results indicated that the α-dTBP antibodies inhibited transcription from the U1TATA and the U1WT templates in a parallel manner (Fig. 5A). To examine the specificity of the inhibition, several additional experiments were performed. First, we preblocked the α-dTBP antibodies by incubating them with bacterially expressed dTBP (purified by Mono S chromatography) prior to adding them to the nuclear extract. As shown in Fig. 5B, the neutralization step decreased the ability of the antibodies to inhibit transcription from both the U1TATA and U1WT templates in a similar manner.

In the next experiment (Fig. 5C), the extract was incubated with antibodies and then adsorbed with protein A-Sepharose. This immunodepletion procedure permanently removes TBP and any TBP-associated factors (TAFs) from the extract (5, 35, 56). Figure 5C shows that the immunodepleted extract was essentially inactive on both the U1TATA and U1WT templates in comparison with the mock-depleted SNF (cf. lanes 2, 3, 6, and 7). Addition of recombinant dTBP restored correct initiation of transcription from the U1TATA promoter (Fig. 5C, lane 4); this result indicates that the inhibition of transcription is due to the specificity of the antibodies for TBP. In contrast to the result with the U1TATA template, recombinant dTBP was unable to restore accurate initiation of transcription from the wild-type U1 promoter (Fig. 5C, lane 8). When hTBP was substituted for dTBP in these experiments, an identical set of results was obtained (i.e., restoration from the U1TATA promoter but not from the U1WT promoter [data not shown]).

The results presented in Fig. 5C are consistent with the findings of other investigators who have likewise analyzed transcriptional activity after supplementing immunodepleted extracts with recombinant TBP. Namely, although recombinant TBP can readily restore transcription from mRNA or U6 RNA promoters that contain TATA boxes, recombinant TBP is unable to restore transcription from mRNA, rRNA, or tRNA promoters that lack TATA boxes (5, 27, 35, 41, 50, 54, 56). The interpretation of those experiments was that TAFs, coimmunodepleted with TBP, are required for recruitment of TBP to TATA-less promoters. Consistent with this interpretation, a partial restoration of accurate transcriptional initiation (albeit at a very low level) was observed on the U1WT as well as on the U1TATA promoter when immunodepleted extract was supplemented with a nuclear fraction containing native TFIID rather than TBP (Fig. 5C, lanes 1 and 5).

A further experiment that took advantage of the fact that the antibodies raised against dTBP do not significantly cross-react with hTBP (35 and our unpublished data) was performed. Nuclear extract was preincubated with α-dTBP antibodies at 4°C for 1 h, and then hTBP was added and incubation was continued for 15 min at 25°C prior to addition of template and NTPs. Figure 5D shows that under these experimental conditions, hTBP can indeed support accurate initiation of transcription from both promoters, although restoration of activity on the U1TATA promoter was still more efficient. Although formal evidence is lacking, a possible explanation of these results is that essential TAFs may

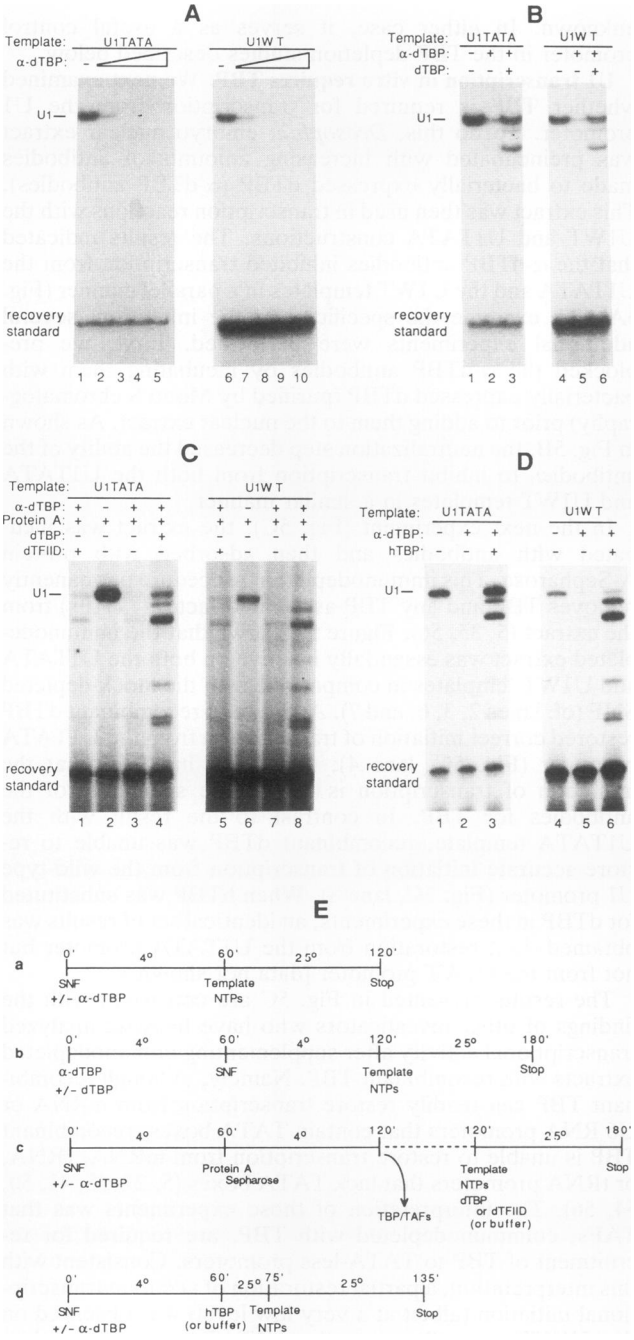

FIG. 5. Transcription from the U1WT and U1TATA promoters is specifically inhibited by antibodies to TBP. (A) Increasing amounts (0, 0.1, 0.3, 0.6, or 1.0 μl) of α-dTBP antibodies were preincubated with the SNF for 1 h at 4°C. rNTPs and the U1TATA (lanes 1 to 5) or the U1WT (lanes 6 to 10) template were then added, and incubation was continued for 1 h at 25°C. (B) α-dTBP antibodies (0.3 μl) were preincubated with buffer (lanes 2 and 5) or with 4 μl of bacterially expressed dTBP (lanes 3 and 6) for 1 h at 4°C. These mixtures were then added to the nuclear extracts, and incubation was continued at 4°C for another hour. In lanes 1 and 4, neither antibodies nor dTBP was added. The treated and untreated extracts were then assayed for transcriptional activity on the U1TATA and the U1WT promoters (left and right panels, respectively). The asterisks denote shorter primer extension products of undefined origin that are enhanced when the reaction mixtures contain added TBP and antibodies. (C) Nuclear extract was preincubated with or without α-dTBP antibodies as indicated for 1 h at 4°C (0.6 μl of antibody per 10 μl of extract). TBP-TAF-antibody complexes were removed by treating the mixtures with protein A-Sepharose for 1 h at 4°C followed by centrifugation. Transcription on the U1TATA (left panel) or on the U1WT (right panel) promoter was carried out by using 12.6 μl of treated extract. Recombinant dTBP (2.5 μl) or partially purified *Drosophila* native TFIID (10 μl) was added as indicated. U1 marks the position of transcripts with the correct start site. (D) Nuclear extract was preincubated with or without α-dTBP antibodies as indicated. Recombinant hTBP was then added to the reaction mixtures shown in lanes 3 and 6, and a further incubation was performed for 15 min at 25°C prior to addition of template and rNTPs. (E) Schematic representations (a, b, c, and d) of the protocols that correspond to the experiments shown in panels A, B, C, and D, respectively. The oligonucleotide used for primer extension in these experiments was a 24-mer that produced an 89-nucleotide primer extension product. The recovery standard was an end-labeled 54-mer. In each experiment shown, the autoradiograms in the right panels (U1WT) are approximately fourfold-longer exposures than the autoradiograms shown in the left panels (U1TATA).

dissociate from the native *Drosophila* TBP complex during the 25°C incubation and then participate in transcription by association with the added hTBP.

Finally, the TBP requirement for transcription from the wild-type U1 promoter was confirmed by using an independent approach. An oligonucleotide that contained the wild-type TATA box sequence of the adenovirus major late promoter was added to the transcription reaction mixtures to sequester TBP prior to addition of the DNA template and NTPs (Fig. 6). Fifty nanograms of this oligonucleotide severely inhibited transcription (Fig. 6, lane 3), and 200 ng was completely inhibitory (lane 4). Addition of a mutant TATA oligonucleotide (Fig. 6, lanes 5 to 7), which was identical except for two base changes that abolish binding to TBP (17), resulted in only a slight (perhaps nonspecific) inhibition of transcription at the highest concentration of oligonucleotide tested. Together, the results presented in Fig. 5 and 6 provide strong evidence that TBP plays an essential role in the transcription of the U1 snRNA gene in vitro.

DISCUSSION

Functional characterization of the U1 promoter in vitro. We have characterized the *cis*-acting elements responsible for accurate and efficient in vitro transcription of a *Drosophila* U1 snRNA gene. As little as 72 bp of 5′-flanking DNA is sufficient to drive an accurate basal level of transcription from the U1 promoter. Within this region, two distinct functional elements have been identified, PSEA and PSEB. PSEA is essential for promoter activity and is the dominant element for specifying the transcription start site. On the basis of these roles and its location in the 5′-flanking DNA, PSEA is probably the functional equivalent of the vertebrate PSE. A potential structural difference, however, is that the *Drosophila* PSEA is a longer sequence than has usually been associated with vertebrate PSEs. Among the PSEAs of 12 *Drosophila* U1, U2, and U4 snRNA genes, 21 bp is well conserved (23), whereas vertebrate PSEs, in general, exhibit a lower degree of sequence conservation that stretches over a shorter distance (7). In several instances, however, mutational effects on transcription have been observed over a comparable 21-bp region surrounding the PSEs of vertebrate snRNA genes (14, 25, 42, 43).

The second conserved element in *Drosophila* snRNA promoters we have termed PSEB. Mutation of PSEB to an

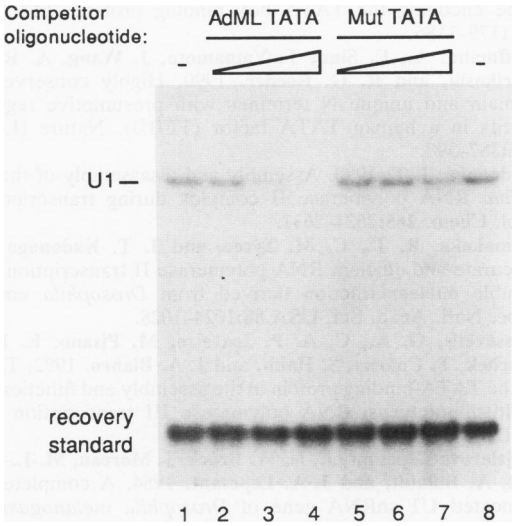

FIG. 6. Transcription of the U1 gene is specifically inhibited by an oligonucleotide containing a consensus TATA box sequence. Increasing amounts of a specific or a control oligonucleotide were preincubated with nuclear extract for 15 min at 25°C prior to addition of the wild-type U1 template and rNTPs. Lanes 2 to 4 contained 15, 50, and 200 ng, respectively, of a double-stranded oligonucleotide possessing the TATA sequence of the adenovirus major late promoter. Lanes 5 to 7 contained 15, 50, and 200 ng, respectively, of an oligonucleotide identical in sequence except for two base changes in the TATA box. No oligonucleotide was added to the reaction mixtures shown in lanes 1 and 8.

unrelated sequence caused an eightfold reduction in template activity in the in vitro assay. In vertebrates, sequences at this position in Pol II-transcribed snRNA genes are not well conserved, and their replacement with unrelated sequences has not been observed to affect transcription in vivo (in transient expression assays) or in vitro (11, 14, 31). However, in sea urchins, at least some Pol II snRNA genes contain a TATA-like sequence near position −25 that is required for efficient expression (47). In plant Pol II snRNA genes there is also a TATA box at this position (51). Although PSEB is not obviously similar to a TATA box, it can be converted to a canonical TATA sequence via three nucleotide transition mutations. These three nucleotide changes were sufficient to increase the basal level of U1 transcription over fourfold and to render the promoter independent of the upstream PSEA sequence, which is otherwise essential for activity.

Since U6 RNA genes throughout the animal and plant kingdoms (including *Drosophila melanogaster*) are transcribed by Pol III and possess canonical TATA sequences near the location of PSEB, we considered the possibility that changing PSEB to a consensus TATA box may have changed the polymerase specificity of the U1 promoter. However, experiments with α-amanitin and tagetitoxin indicated that the Pol II specificity was unaltered. On the basis of published results from other systems (22, 26, 51), we suspect that in the U1TATA promoter the spacing between the PSEA and the TATA sequence is incompatible with recognition by Pol III. In the 5′-flanking DNA of *Drosophila* U6 genes, a PSEA-like sequence is located about 2 to 3 bp further upstream of the transcription start site relative to its position in Pol II snRNA gene promoters, and the U6 TATA box is 1 to 3 bp closer to the start site than is the PSEB sequence (9). As a result, the distance between PSEA and PSEB in *Drosophila* Pol II snRNA promoters is 3 to 6 bp less than the distance between the PSEA-like sequence and the TATA box of *Drosophila* U6 promoters. This is reminiscent of the situation in vertebrates, in which a 4-bp upstream displacement of the PSE is an important determinant of Pol III specificity (22).

Role of TBP in U1 transcription. Recent work from several laboratories has, surprisingly, revealed that TBP is required for transcription (i) by Pol II from mRNA promoters that contain or lack TATA boxes (2, 3, 35), (ii) by Pol I from rRNA promoters (5, 6, 40), and (iii) by Pol III from 5S, tRNA, and U6 RNA promoters (6, 20, 26, 27, 40–42, 50, 54, 55). The results presented here now demonstrate that TBP is also required for transcription of the U1 snRNA gene by Pol II. This essentially completes the story that TBP is required for transcription from all known classes and distinct types of promoters.

TBP is a component of the classical transcription factor activities known as SL1, TFIID, and TFIIIB that are utilized by Pol I, II, and III, respectively (38). The particular functional activity exhibited by TBP is dependent upon unique sets of TAFs that are associated with TBP in distinct multiprotein complexes. From the results presented in Fig. 5C and D, transcription of the U1 gene is apparently dependent upon a TBP-TAF complex, since supplementation of an immunodepleted extract with recombinant dTBP could not restore transcription from the wild-type U1 promoter, whereas transcription from the U1TATA promoter was readily restored. Moreover, supplementation of immunodepleted extracts with a fraction containing native TFIID restored a detectable level of correct U1 initiation (Fig. 5C, lanes 1 and 4). However, since the level of restoration was very low, and since the native *Drosophila* TFIID fraction was not homogeneous, we cannot be certain whether TFIID (as defined for mRNA promoters) or a unique TBP complex (that partially cofractionates with TFIID) participates in U1 snRNA transcription. Interestingly, we have found that a fractionated *Drosophila* transcription system that accurately and efficiently transcribes mRNA promoters (10, 53) is incapable of transcribing either the wild-type U1 promoter or the U1TATA promoter (50a). Thus, the U1TATA promoter, as well as the wild-type promoter, may require unique factors for initiation of transcription. The *Drosophila* SNF should be an excellent source of material for the biochemical purification and characterization of these factors. In future work, it will be interesting to examine and compare the compositions of the TBP complexes involved in mRNA, U1 snRNA (Pol II), and U6 snRNA (Pol III) transcription.

Although our data do not rule out that PSEB may be a stimulatory element unrelated to the TATA box and recognized by an unknown sequence-specific transactivator protein, it is reasonable to speculate that PSEB is the site of interaction with the TBP complex that is required for U1 transcription. If this is true, TBP may recognize or strongly interact with PSEB only when a second protein is first bound to the upstream PSEA sequence. In such a model, the PSEA-binding protein would by some mechanism facilitate or stabilize the binding of TBP to the PSEB sequence, probably through interactions with TAFs as intermediates or coactivators. Indeed, PSEB may represent a noncanonical TATA box that forces or requires the U1 promoter to be dependent upon the upstream PSEA. In the case of snRNA genes, this may be part of a mechanism to ensure that snRNA, rather than mRNA, Pol II preinitiation complexes are formed on snRNA gene promoters. During the evolution

of vertebrates, an equivalent to the PSEB sequence may have become dispensable for snRNA transcription by Pol II, with all of the essential functions being accomplished via protein-protein interactions. Alternatively, sequences in the −30 region of vertebrate Pol II snRNA genes may play a subtle role in vivo that is not readily apparent in transient-expression assays.

ACKNOWLEDGMENTS

We thank Jim Kadonaga and Sanford Bernstein for helpful discussions and for a critical reading of the manuscript prior to submission. We also thank Kathleen McNamara for preparation of plasmid DNA and additional technical assistance.

This work was supported by Public Health Service grant GM-33512 from the National Institutes of Health to W.E.S. and in part by the California Metabolic Research Foundation. C.M.T. is supported by Public Health Service grant GM-41249 to J. Kadonaga.

REFERENCES

1. **Beck, E., J. L. Jorcano, and A. Alonso.** 1984. *Drosophila melanogaster* U1 and U2 small nuclear RNA genes contain common flanking sequences. J. Mol. Biol. **173**:539–542.
2. **Bungert, J., I. Kober, F. Düring, and K. H. Seifart.** 1992. Transcription factor eUSF is an essential component of isolated transcription complexes on the duck histone H5 gene and it mediates the interaction of TFIID with a TATA-deficient promoter. J. Mol. Biol. **223**:885–898.
3. **Carcamo, J., L. Buckbinder, and D. Reinberg.** 1991. The initiator directs the assembly of a transcription factor IID-dependent transcription complex. Proc. Natl. Acad. Sci. USA **88**:8052–8056.
4. **Chu, B. C. F., and L. E. Orgel.** 1991. Binding of hairpin and dumbbell DNA to transcription factors. Nucleic Acids Res. **19**:6958.
5. **Comai, L., N. Tanese, and R. Tjian.** 1992. The TATA-binding protein and associated factors are integral components of the RNA polymerase I transcription factor, SL1. Cell **68**:965–976.
6. **Cormack, B. P., and K. Struhl.** 1992. The TATA-binding protein is required for transcription by all three nuclear RNA polymerases in yeast cells. Cell **69**:685–696.
7. **Dahlberg, J. E., and E. Lund.** 1988. The genes and transcription of the major small nuclear RNAs, p. 38–70. *In* M. L. Birnstiel (ed.), Structure and function of major and minor small nuclear ribonucleoprotein particles. Springer Verlag KG, Heidelberg, Germany.
8. **Dahlberg, J. E., and E. Lund.** 1991. How does III × II make U6? Science **254**:1462–1463.
9. **Das, G., D. Henning, and R. Reddy.** 1987. Structure, organization, and transcription of Drosophila U6 small nuclear RNA genes. J. Biol. Chem. **262**:1187–1193.
10. **Dynlacht, B. D., T. Hoey, and R. Tjian.** 1991. Isolation of coactivators associated with the TATA-binding protein that mediate transcriptional activation. Cell **66**:563–576.
11. **Gunderson, S. I., M. W. Knuth, and R. R. Burgess.** 1990. The human U1 snRNA promoter correctly initiates transcription in vitro and is activated by PSE1. Genes Dev. **4**:2048–2060.
12. **Guthrie, C.** 1991. Messenger RNA splicing in yeast: clues to why the spliceosome is a ribonucleoprotein. Science **253**:157–163.
13. **Heiermann, R., and O. Pongs.** 1985. *In vitro* transcription with extracts of nuclei of *Drosophila* embryos. Nucleic Acids Res. **13**:2709–2730.
14. **Hernandez, N., and R. Lucito.** 1988. Elements required for transcription initiation of the human U2 snRNA gene coincide with elements required for snRNA 3′ end formation. EMBO J. **7**:3125–3134.
15. **Hernandez, N., and A. M. Weiner.** 1986. Formation of the 3′ end of U1 snRNA requires compatible snRNA promoter elements. Cell **47**:249–258.
16. **Hoey, T., B. D. Dynlacht, M. G. Peterson, B. F. Pugh, and R. Tjian.** 1990. Isolation and characterization of the Drosophila gene encoding the TATA box binding protein, TFIID. Cell **61**:1179–1186.
17. **Hoffmann, A., E. Sinn, T. Yamamoto, J. Wang, A. Roy, M. Horikoshi, and R. G. Roeder.** 1990. Highly conserved core domain and unique N terminus with presumptive regulatory motifs in a human TATA factor (TFIID). Nature (London) **346**:387–390.
18. **Kadonaga, J. T.** 1990. Assembly and disassembly of the *Drosophila* RNA polymerase II complex during transcription. J. Biol. Chem. **265**:2624–2631.
19. **Kamakaka, R. T., C. M. Tyree, and J. T. Kadonaga.** 1991. Accurate and efficient RNA polymerase II transcription with a soluble nuclear fraction derived from *Drosophila* embryos. Proc. Natl. Acad. Sci. USA **88**:1024–1028.
20. **Kassavetis, G. A., C. A. P. Joazeiro, M. Pisano, E. P. Geiduschek, T. Colbert, S. Hahn, and J. A. Blanco.** 1992. The role of the TATA-binding protein in the assembly and function of the multisubunit yeast RNA polymerase III transcription factor, TFIIIB. Cell **71**:1055–1064.
21. **Kejzlarova-Lepesant, J., H. W. Brock, J. Moreau, M.-L. Dubertret, A. Billault, and J.-A. Lepesant.** 1984. A complete and a truncated U1 snRNA gene of *Drosophila melanogaster* are found as inverted repeats at region 82E of the polytene chromosome. Nucleic Acids Res. **12**:8835–8846.
22. **Lescure, A., P. Carbon, and A. Krol.** 1991. The different positioning of the proximal sequence element in the Xenopus RNA polymerase II and III snRNA promoters is a key determinant which confers RNA polymerase III specificity. Nucleic Acids Res. **19**:435–441.
23. **Lo, P. C. H., and S. M. Mount.** 1990. *Drosophila melanogaster* genes for U1 snRNA variants and their expression during development. Nucleic Acids Res. **18**:6971–6979.
24. **Lobo, S. M., and N. Hernandez.** 1989. A 7 bp mutation converts a human RNA polymerase II snRNA promoter into an RNA polymerase III promoter. Cell **58**:55–67.
25. **Lobo, S. M., S. Ifill, and N. Hernandez.** 1990. *Cis*-acting elements required for RNA polymerase II and III transcription in the human U2 and U6 snRNA promoters. Nucleic Acids Res. **18**:2891–2899.
26. **Lobo, S. M., J. Lister, M. L. Sullivan, and N. Hernandez.** 1991. The cloned RNA polymerase II transcription factor IID selects RNA polymerase III to transcribe the human U6 gene in vitro. Genes Dev. **5**:1477–1489.
27. **Lobo, S. M., M. Tanaka, M. L. Sullivan, and N. Hernandez.** 1992. A TBP complex essential for transcription from TATA-less but not TATA-containing RNA polymerase III promoters is part of the TFIIIB fraction. Cell **71**:1029–1040.
28. **Lund, E., and J. E. Dahlberg.** 1989. In vitro synthesis of vertebrate U1 snRNA. EMBO J. **8**:287–292.
29. **Mattaj, I. W., N. A. Dathan, H. D. Parry, P. Carbon, and A. Krol.** 1988. Changing the RNA polymerase specificity of U snRNA gene promoters. Cell **55**:435–442.
30. **Morris, G. F., D. H. Price, and W. F. Marzluff.** 1986. Synthesis of U1 RNA in a DNA-dependent system from sea urchin embryos. Proc. Natl. Acad. Sci. USA **83**:3674–3678.
31. **Murphy, J. T., J. M. Skuzeski, E. Lund, T. H. Steinberg, R. R. Burgess, and J. E. Dahlberg.** 1987. Functional elements of the human U1 RNA promoter. III. Identification of five separate regions required for efficient transcription and template competition. J. Biol. Chem. **262**:1795–1803.
32. **Neuman de Vegvar, H. E., and J. E. Dahlberg.** 1989. Initiation and termination of human U1 RNA transcription requires the concerted action of multiple flanking elements. Nucleic Acids Res. **17**:9305–9318.
33. **Neuman de Vegvar, H. E., E. Lund, and J. E. Dahlberg.** 1986. 3′ end formation of U1 snRNA precursors is coupled to transcription from snRNA promoters. Cell **47**:259–266.
34. **Parry, H. D., D. Scherly, and I. W. Mattaj.** 1989. Snurpogenesis: the transcription and assembly of U snRNP components. Trends Biochem. Sci. **14**:15–19.
35. **Pugh, B. F., and R. Tjian.** 1991. Transcription from a TATA-less promoter requires a multisubunit TFIID complex. Genes Dev. **5**:1935–1945.

36. **Reddy, R.** 1988. Transcription of a U6 small nuclear RNA gene in vitro. J. Biol. Chem. **263**:15980–15984.
37. **Reddy, R., and R. Singh.** 1991. Synthesis of small nuclear RNAs, p. 1–36. *In* P. Jeanteur, Y. Kuchino, W. E. G. Muller, and P. L. Paine (ed.), Progress in molecular and subcellular biology. Springer-Verlag, Berlin.
38. **Rigby, P. W. J.** 1993. Three in one and one in three: it all depends on TBP. Cell **72**:7–10.
39. **Saba, J. A., H. Busch, D. Wright, and R. Reddy.** 1986. Isolation and characterization of two putative full-length *Drosophila* U4 small nuclear RNA genes. J. Biol. Chem. **261**:8750–8753.
40. **Schultz, M. C., R. H. Reeder, and S. Hahn.** 1992. Variants of the TATA-binding protein can distinguish subsets of RNA polymerase I, II, and III promoters. Cell **69**:697–702.
41. **Simmen, K. A., J. Bernués, J. D. Lewis, and I. W. Mattaj.** 1992. Cofractionation of the TATA-binding protein with the RNA polymerase III transcription factor TFIIIB. Nucleic Acids Res. **20**:5889–5898.
42. **Simmen, K. A., J. Bernués, H. D. Parry, H. G. Stunnenberg, A. Berkenstam, B. Cavallini, J.-M. Egly, and I. W. Mattaj.** 1991. TFIID is required for *in vitro* transcription of the human U6 gene by RNA polymerase III. EMBO J. **10**:1853–1862.
43. **Simmen, K. A., R. Waldschmidt, J. Bernués, H. D. Parry, K. H. Seifart, and I. W. Mattaj.** 1992. Proximal sequence element factor binding and species specificity in vertebrate U6 snRNA promoters. J. Mol. Biol. **223**:873–884.
44. **Sluder, A. E., D. H. Price, and A. L. Greenleaf.** 1987. An activity necessary for *in vitro* transcription is a DNase inhibitor. Biochimie **69**:1199–1205.
45. **Soeller, W. C., S. J. Poole, and T. Kornberg.** 1988. *In vitro* transcription of the *Drosophila engrailed* gene. Genes Dev. **2**:68–81.
46. **Southgate, C., and M. Busslinger.** 1989. *In vivo* and *in vitro* expression of U7 snRNA genes: cis- and trans-acting elements required for RNA polymerase II-directed transcription. EMBO J. **8**:539–549.
47. **Stefanovic, B., and W. F. Marzluff.** 1992. Characterization of two developmentally regulated sea urchin U2 small nuclear RNA promoters: a common required TATA sequence and independent proximal and distal elements. Mol. Cell. Biol. **12**:650–660.
48. **Steinberg, T. H., D. E. Mathews, R. D. Durbin, and R. R. Burgess.** 1990. Tagetitoxin: a new inhibitor of eukaryotic transcription by RNA polymerase III. J. Biol. Chem. **265**:499–505.
49. **Steitz, J. A., D. L. Black, V. Gerke, K. A. Parker, A. Kramer, D. Frendewey, and W. Keller.** 1988. Functions of the abundant U-snRNPs, p. 115–154. *In* M. L. Birnsteil (ed.), Structure and function of major and minor small nuclear ribonucleoprotein particles. Springer Verlag KG, Heidelberg, Germany.
50. **Taggart, A. K. P., T. S. Fisher, and B. F. Pugh.** 1992. The TATA-binding protein and associated factors are components of Pol III transcription factor TFIIIB. Cell **71**:1015–1028.
50a. **Tyree, C. M.** Unpublished data.
51. **Waibel, F., and W. Filipowicz.** 1990. RNA-polymerase specificity of transcription of Arabidopsis U snRNA genes determined by promoter element spacing. Nature (London) **346**:199–202.
52. **Waldschmidt, R., I. Wanandi, and K. H. Seifart.** 1991. Identification of transcription factors required for the expression of mammalian U6 genes in vitro. EMBO J. **10**:2595–2603.
53. **Wampler, S. L., C. M. Tyree, and J. T. Kadonaga.** 1990. Fractionation of the general RNA polymerase II transcription factors from Drosophila embryos. J. Biol. Chem. **265**:21223–21231.
54. **White, R. J., and S. P. Jackson.** 1992. Mechanism of TATA-binding protein recruitment to a TATA-less class III promoter. Cell **71**:1041–1053.
55. **White, R. J., S. P. Jackson, and P. W. J. Rigby.** 1992. A role for the TATA-box-binding protein component of the transcription factor IID complex as a general RNA polymerase III transcription factor. Proc. Natl. Acad. Sci. USA **89**:1949–1953.
56. **Zhou, Q., P. M. Lieberman, T. G. Boyer, and A. J. Berk.** 1992. Holo-TFIID supports transcriptional stimulation by diverse activators and from a TATA-less promoter. Genes Dev. **6**:1964–1974.